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# Steroid signalling in human ovarian surface epithelial cells: the response to interleukin-1 $\alpha$ determined by microarray analysis

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## Abstract

The human ovarian surface epithelium (HOSE) is a common site of gynaecological disease including endometriosis and ovarian cancer, probably due to serial injury-repair events associated with successive ovulations. To comprehend the importance of steroid signalling in the regulation of the HOSE, we used a custom microarray to catalogue the expression of over 250 genes involved in the synthesis and reception of steroid hormones, sterols and retinoids. The array included a subset of non-steroidogenic genes commonly involved in pro-/anti-inflammatory signalling. HOSE cells donated by five patients undergoing surgery for non-malignant gynaecological conditions were cultured for 48 h in the presence and absence of 500 pg/ml interleukin-1 $\alpha$  (IL-1 $\alpha$ ). Total RNA was reverse-transcribed into biotin-labelled cDNA, which was hybridised to the array and visualised by gold-particle resonance light scattering and charge-coupled device (CCD) camera detection. Results for selected genes were verified by quantitative reverse-transcription PCR. In five out of five cases, untreated HOSE cells expressed genes encoding enzymes required for *de novo* biosynthesis of cholesterol from acetate and subsequent formation of C21-pregnane and C19-androstane steroids. Consistent with the inability

of HOSE cells to synthesise glucocorticoids, oestrogens or 5 $\alpha$ -reduced androgens *de novo*, CYP21, CYP19 and 5 $\alpha$ -reductase were not detected. The only steroidogenic gene significantly up-regulated by IL-1 $\alpha$  was 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ HSD1). Other cytokine-induced genes were IL-6, IL-8, nuclear factor  $\kappa$ B (NF $\kappa$ B) inhibitor  $\alpha$ , metallothionein-IIA and lysyl oxidase: inflammation-associated genes that respond to glucocorticoids. The only steroidogenic gene significantly suppressed by IL-1 $\alpha$  was 3 $\beta$ HSD1. Other genes suppressed by IL-1 $\alpha$  were aldehyde dehydrogenase (ALDH) 1, ALDH 10, gonadotrophin hormone-releasing hormone receptor, peroxisome proliferation-activated receptor-binding protein (PPAR-bp) and nuclear receptor subfamily 2 group F member 2. These results define a steroidogenic phenotype of cultured HOSE cells and provide a limited expression profile for genes with associated signalling functions. IL-1 $\alpha$  co-ordinately induces 11 $\beta$ HSD1 and a panel of glucocorticoid-regulated, inflammation-associated genes in HOSE cells, providing further evidence that cortisol generated by 11 $\beta$ HSD1 could participate in the local resolution of inflammation associated with ovulation.

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## Introduction

The steroid-secretory function of the ovaries is well recognised but the capacity of the human ovarian surface epithelium (HOSE) to synthesise, metabolise and respond to steroids remains obscure. The probable importance of steroid signalling in the HOSE relates to the tendency of this cellular layer to undergo neoplastic transformation and produce fatal tumours. Ovarian cancers represent approximately 4% of all gynaecological cancers and are the fifth highest cause of death from cancer among women. Over 90% of ovarian cancers in women are believed to originate in the HOSE. Many such cancers are steroid responsive

and one of the major negative-risk factors is anovulation associated with steroidal oral contraceptive usage. HOSE cells are believed to be steroid responsive on the basis that exposure to ovarian steroids *in vitro* affects HOSE cell proliferation (Karlan *et al.* 1995) and that they express nuclear hormone receptors, including oestrogen receptor (ER; Hillier *et al.* 1998, Lau *et al.* 1999, Li *et al.* 2003), androgen receptor (AR; Lau *et al.* 1999, Edmondson *et al.* 2002) and progesterone receptor (PR; Karlan *et al.* 1995, Lau *et al.* 1999, Li *et al.* 2003), which presumably mediate these effects. Involvement of HOSE cells in steroid biosynthesis is less certain. However, definitive evidence for steroid metabolism comes from the recent discovery

that cultured HOSE cells express the glucocorticoid-metabolising enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ HSD1), which is up-regulated by exposure to interleukin-1 $\alpha$  (IL-1 $\alpha$ ) (Yong *et al.* 2002, Rae *et al.* 2004). Pro-inflammatory cytokines such as IL-1 $\alpha$  are increasingly produced by follicular cells at the time of ovulation, therefore up-regulation of 11 $\beta$ HSD1 could be a mechanism for increasing local metabolism of substrate cortisone to cortisol and promoting anti-inflammatory glucocorticoid action at the ovarian surface (Hillier & Tetsuka 1998).

To define the steroidogenic signature of the HOSE we developed a custom gene microarray probing the enzymes that catalyse the complete steroidogenic pathway, from acetate through cholesterol to the principal hormonal steroids and their metabolites. The gene-set also contains nuclear receptors of known and unknown functions and a limited selection of genes encoding pro-/anti-inflammatory mediators. Here we apply this microarray to analyse RNA from primary HOSE cell cultures, with and without prior exposure of cells to IL-1 $\alpha$ . The results define a steroidogenic phenotype of normal HOSE cells and identify a panel of associated HOSE genes with likely roles in normal and abnormal ovarian function. Several such genes are glucocorticoid responsive and are likely to be involved in the injury-repair process associated with ovulation.

## Materials and Methods

### Patients

HOSE cells were obtained (with informed consent after local ethics committee approval) from normal ovaries of eight premenopausal women undergoing surgery for non-malignant gynaecological conditions. Cells were collected at laparotomy by gentle scraping of the ovarian surface with a sterile wooden spatula, which was then rinsed into sterile, pre-warmed, culture medium (see below). Cells were collected as near as practicable to the beginning of the surgical procedure to avoid any contamination with blood cells. Collections were then examined by phase-contrast microscopy to verify that a representative biopsy of the HOSE had been recovered.

### Production of HOSE cell monolayers

All cultures were produced and handled in an identical manner, using reagents made from a common stock. The tissue culture reagents were obtained from Gibco and Sigma. HOSE scrapings were cultured in donor calf serum-precoated flasks (75 cm<sup>2</sup>; Corning Inc, Glass Works, Corning, NY, USA). Culture medium (HOSE 1) consisted of Medium199:MCDB105 (1:1 v/v) supplemented with fetal calf serum (15% v/v), streptomycin

(50  $\mu$ g/ml), penicillin (50 IU/ml) and L-glutamine (2 mM) (Yong *et al.* 2002). Cells were incubated at 37 °C in a humidified incubator under an atmosphere of 95% air–5% CO<sub>2</sub> for 21 days, with medium renewed every 7 days. HOSE cell monolayers were routinely examined by phase-contrast microscopy for contaminating fibroblasts. Cell purity was checked in selected cases by immunocytochemical staining for cytokeratin 5, 6, 8 and 17 (Auersperg *et al.* 2001) using a commercially available monoclonal anti-human cytokeratin antibody (Dako, Ely, Cambridgeshire, UK), which confirmed that the monolayers comprised pure epithelial cells (data not shown).

### Experimental treatment of cultured HOSE cells

Each set of experiments was applied identically to cells donated by five different patients. HOSE cell monolayers (21 days old) were dispersed by treatment with trypsin-EDTA in Hanks' balanced salt solution (0.05% w/v trypsin, 0.5 mM EDTA, Invitrogen) for exactly 5 min at 37 °C. Cells were collected by centrifugation for 5 min at 800 g, and the pellet was washed once and re-suspended in fresh HOSE 1 medium. Cell counts were done with a haemocytometer using trypan blue (Sigma) dye-exclusion to determine viability (75–90%). The cell suspension was adjusted to a concentration of 100 000 cells/ml with HOSE 1 medium and 15 ml portions dispersed into fresh 75 cm<sup>2</sup> culture flasks, which were incubated for 24 h at 37 °C to allow cell attachment. The medium was then replaced with serum-depleted medium (HOSE 2) containing 0.01% BSA (w/v) and incubation continued for a further 24 h. Spent medium was then replaced with fresh HOSE 2 containing no addition (control) or 500 pg/ml recombinant human IL-1 $\alpha$  (R&D Systems Europe Ltd, Abingdon, Oxon, UK), with incubation for 48 h at 37 °C. These conditions have previously been shown to be optimal for inducing 11 $\beta$ HSD enzymatic activity in cultured HOSE cells (Yong *et al.* 2002, Rae *et al.* 2004). Total RNA was then extracted from the cell monolayers as described below. Additional experiments to establish the effects of cortisol on IL-1 $\alpha$ -induced lysyl oxidase (LOX) gene expression were performed as previously described (Rae *et al.* 2004), using primary HOSE cell cultures donated by a further three patients.

### RNA extraction and quality analysis

RNA was extracted using RNeasy minispinn columns (Qiagen) as per manufacturer's protocols, in combination with on-column DNase-I treatment (Qiagen). Aliquots (1  $\mu$ l) of purified RNA were removed for quantification and quality assessment of total RNA using the Agilent 2100 Bioanalyser system in combination with RNA<sub>6000</sub> nano chips (Agilent Technologies, Cheshire, UK). Only RNA that displayed intact 18S and 28S peaks

was reverse transcribed (RT) to cDNA for real-time PCR analysis. This quality-control step was included for each experimental run to avoid generation of false-negative results due to RNA degradation before and during extraction steps, and also as a quantification method to ensure equal amounts of RNA were transcribed in each RT reaction.

#### *EUROSTERONE microarray fabrication*

The gene sets used on the microarray were chosen by the European Commission funded EUROSTERONE consortium (Hillier 2003), comprising 256 genes involved in steroid signalling, including enzymes of the major biochemical pathways of cholesterol and steroid hormone biosynthesis, nuclear hormone receptors and a subset of associated genes implicated in pro-/anti-inflammatory signalling. Fifty-mer oligonucleotides specific for each gene were selected in-house and synthesised by MWG (Milton Keynes, UK). Oligonucleotides were dissolved in 75 mM sodium-phosphate buffer containing 0.005% (w/v) SDS. Plates of oligonucleotide solutions were prepared using a Roboamp4200 (MWG) and spotted in triplicate on to Corning GAPS II slides (25 × 27 mm bar-coded, lot. 34802000B, Corning, Netherlands) using a MG2 array spotter (Biorobotics, Cambridgeshire, UK) in conjunction with 200 µm solid pins, producing a spot diameter of 310–330 µm, under temperature and humidity conditions of 16–18 °C and 35–45% respectively. After spotting was complete and slides had dried, oligonucleotide spots were covalently linked to the aminosilane slide coating by exposure to u.v. light (250 mJ) (GS Genelinker UV chamber, BioRad). The arrays were stored desiccated at room temperature for use within 2 weeks of preparation to avoid any potential decline in sensitivity.

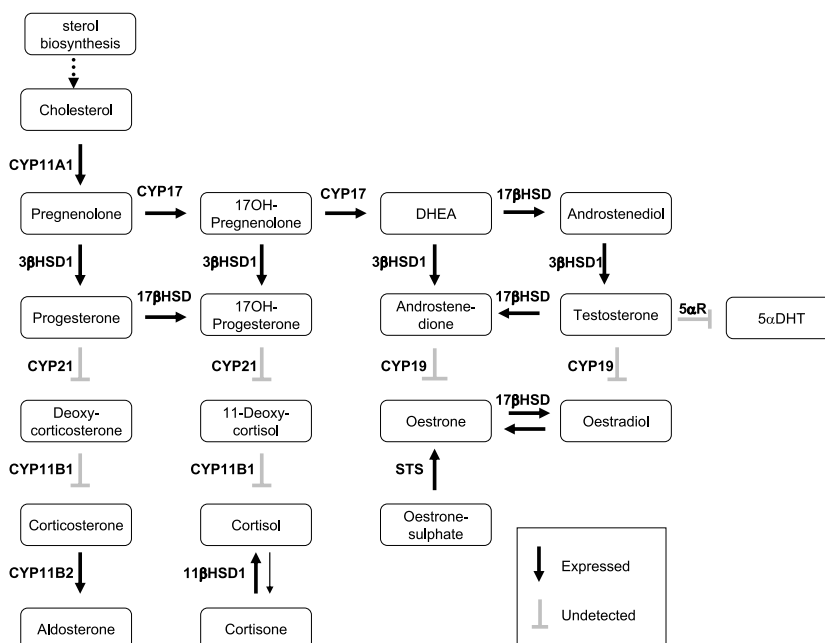
#### *Preparation of labelled cDNA and hybridisation*

Biotin-*d*-11-UTP (Roche) was incorporated into cDNA during reverse transcription using a Labelstar kit as per manufacturer's instructions (Qiagen); in each case using exactly 6 µg (as measured by an Agilent Bioanalyser, and confirmed by spectrophotometry) Dnase-treated RNA. The protocol used in the Labelstar kit can be used with 0.5–50 µg RNA template. An advantage of this method is the endogenous exonuclease activity of the reverse transcriptase enzyme preparation, which avoids unnecessary lysis of RNA template by harsh reagent conditions. cDNA was purified from reaction mixtures by silica-gel binding columns (Qiagen). Hybridisation of exactly 50% of the cDNA produced (equivalent to 3 µg RNA template) was carried out for 18 h at 42 °C in a humidified hybridisation oven using reagents from a Qiagen HiLight single-colour (gold particles) array detection hybridisation kit. The

hybridisation chambers used were single-slide, rubber-sealed units (Camlab, Cambridge, UK). Post-hybridisation washes and detection by addition of anti-biotin serum labelled with colloidal gold was performed as described by the manufacturer's (Qiagen) protocols, except that solution removal was done by centrifugation and the volume of each kit component was adjusted for use with the smaller print area of the array used in this study. In pilot studies, we found that these methods of hybridisation and subsequent detection by gold-particle resonance-light scattering and charge-coupled device (CCD) camera detection were >10-fold more sensitive than conventional Cy5-3/5 labelling and detection strategies as regards signal intensity and amount of template RNA required. We ascertained using the resonance-light scattering system that negligible differences in results occurred using between 1 and 5 µg RNA-templated cDNA, and greater amounts of RNA template were found to be deleterious to array detection. A separate chip was used to analyse each sample (i.e. control and treated samples from each patient were run on separate chips), thus avoiding the need for dye/metal particle-swap experiments.

#### *Scanning and analysis*

Each array was scanned eight times at increasing CCD camera exposure times (there is no photo-bleaching effect with this type of detection). Bitmap scan files were then quantified using Quantarray (Perkin-Elmer, Chalfont, Bucks, UK), using fixed-circle detection parameters, and the resulting comma separated value (CSV) format data exported to Microsoft Excel for manipulation and analysis. Scatter-plots of each scanner setting were created and used to identify which CCD setting gave the optimal dataset for each array, as previously described (Forster *et al.* 2003). Optimal scanner data for each array were then normalised to the mean 75th percentile of total array signal (global normalisation, as previously described (Forster *et al.* 2003). As a data-quality filter, the sensitivity threshold for each array was taken as the 80th percentile of 15 negative-control genes. Values below this number were removed from the dataset for that particular array. In rare cases where genes were either newly induced or completely suppressed by treatment, sub-sensitivity threshold values were analysed to permit statistical analysis of treatment effects. Data were logarithmically transformed to base 2, then further analysed with a *t*-test for dependent samples (paired, two-way *t*-test) on a per-gene basis, utilising the statistical dependence between the treated and untreated samples per patient to best effect. Statistical sample size was limited by patient numbers, therefore consistent with our hypothesis-driven approach, analysis was limited to an 'interest-filter' approach whereby genes were selected on their significance levels (cut-off for statistical significance was set at  $\alpha=0.05$ ). Permutation testing was used to confirm or refute these analyses, and a subset of



**Figure 1** Steroidogenic signature of cultured HOSE cells. Schematic summary of potential for *de novo* steroid synthesis and metabolism under basal conditions of culture, based upon EUROSTERONE custom microarray analysis.

genes significantly modulated by treatment were further validated by real-time PCR analysis (below).

### Real-time PCR analysis

DNase-treated (Qiagen) RNA (200 ng) was reverse transcribed (RT; random hexamer kit; Applied Biosystems, Warrington, UK) and 2 µl of the resultant RT-mix analysed. cDNA was analysed in a 25 µl final assay volume containing 300 nM primers and 200 nM TaqMan hybridisation probe (Biosource UK Ltd, Essex, UK). Primers and probes were designed using Primer-Express software (Perkin-Elmer, Boston, MA, USA) and validated prior to use (11βHSD1 primers and probe; Yong *et al.* 2002), or were purchased pre-validated from Applied Biosystems (Assay-on-Demand systems). Target mRNA was quantified in relation to 18S ribosomal RNA abundance in each sample. Negative controls consisted of RT-negative (RNA template with no reverse transcriptase enzyme), and RT-H<sub>2</sub>O (water in place of RNA template) samples generated at the time of reverse transcription of samples, plus a TaqMan-reaction negative control where cDNA was replaced with water. In the case of the experiments examining the effects of glucocorticoids on IL1α-modulated genes (LOX), this was performed as previously described for a range of other genes in HOSE cells (Rae *et al.* 2004), using a 48 h treatment-incubation time in all cases. Data were logarithmically transformed to base 10

and then analysed by ANOVA with Student's *t*-test to determine specific treatment effects.

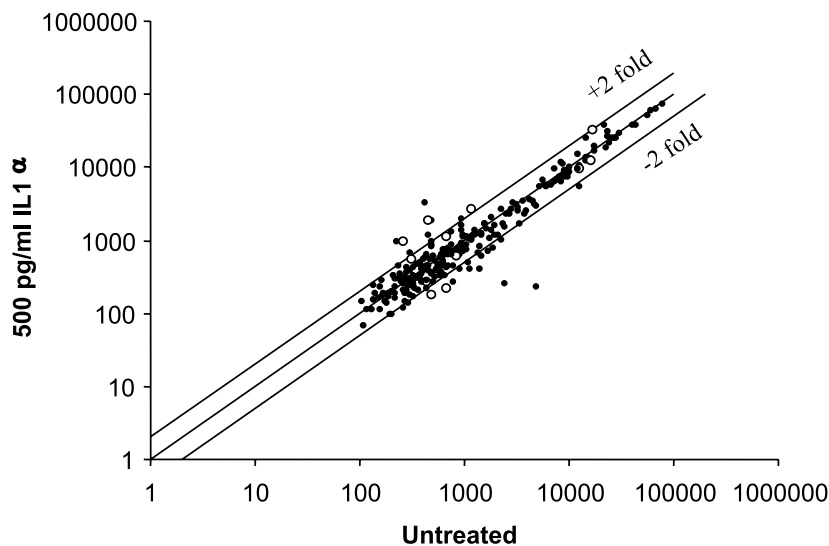
## Results

Untreated HOSE cells from all five patients expressed genes encoding many of the enzymes involved in classic pathways of steroid biosynthesis metabolism and action. The full normalised signature dataset is provided as a supplementary table to the online version of Journal of Endocrinology (see <http://joe.endocrinology-journals.org/content/vol183/issue1/>). In addition, original data as well as selected data not shown in the manuscript are available (accession no. GXE-00021), in the MIAME compliant GTI expression pathway database, GPXdb- (<http://www.gti.ed.ac.uk>).

### Steroid biosynthesis

All the enzymes required for *de novo* formation of C21-pregnane and C19-androstane steroids from acetate via cholesterol were significantly detected. However, CYP21 and CYP19 were not expressed at levels above the sensitivity threshold of the array, suggesting that HOSE cells are unlikely to be able to undertake *de novo* synthesis of biologically active glucocorticoids or oestrogens (Fig. 1).





**Figure 2** Regression plot of global effects of IL-1 $\alpha$  treatment (500 pg/ml, 48 h duration) on primary cultures derived from five separate women (mean of five experiments). Upper line indicates 2-fold up-regulation, lower line indicates 2-fold down-regulation, middle line indicates no regulation by IL-1 $\alpha$ . Open circles represent genes whose expression profiles were found to be significantly different over the  $n=5$  population between untreated and IL-1 $\alpha$ -treated cultures.

#### Steroid-metabolising enzymes

Relatively abundant expression of many key steroid-metabolising enzymes was apparent, including members of the steroid dehydrogenase/reductase (SDR) gene family responsible for inter-conversion of active and inert congeners of hormonal steroids: 3 $\beta$ HSD1, 11 $\beta$ HSD1 and 17 $\beta$ HSD types 1, 2, 4 and 7. The testis-specific 17 $\beta$ HSD isoform, 17 $\beta$ HSD3, was not expressed. Steroid 5 $\alpha$ -reductase types 1 and 2 were also not expressed above sensitivity threshold levels (although steroid 5 $\beta$ -reductase was), implying an inability of HOSE cells to produce 5 $\alpha$ -reduced androgens. Enzymes (CYP11B1 and CYP11B2) required for the metabolism of glucocorticoids to the mineralocorticoid aldosterone were present, as were those involved in the formation and hydrolysis of conjugated sex steroids. These last included steroid sulphatase, which converts oestrone-sulphate into oestrone. Oestrogen sulphotransferase which catalyses the reverse reaction, was not expressed.

#### Nuclear receptors

Nuclear receptors for oestrogens (ER $\alpha$  and ER $\beta$ ), progesterone (PR), glucocorticoids (GR) and androgens (AR) were all present. Other nuclear receptor and related genes expressed by HOSE cells included: retinoic acid receptor (RAR) $\alpha$ , RAR $\beta$ 4 and RAR $\gamma$ ; thyroid hormone receptors (TR $\alpha$ 2); peroxisome-proliferation-activated receptors (PPAR) $\delta$ , PPAR $\gamma$ 2 and related proteins, PPAR co-activator 1 and PPAR-binding protein (transcription

coactivator PBP); heat-shock protein 90 (Hsp90) and a range of orphan nuclear receptors.

#### Responsiveness to IL-1 $\alpha$

Of the core genes on the microarray directly involved in steroid signalling, only one (11 $\beta$ HSD1) was stimulated and another (3 $\beta$ HSD1) inhibited by treatment with IL-1 $\alpha$ . A total of 12 genes were significantly affected by treatment with IL-1 $\alpha$  (Fig. 2). The affected genes are listed in Tables 1 and 2, showing positive and negative responders respectively.

#### Verification by PCR

RT-PCR was performed to verify that the observed changes in gene expression level, determined by microarray, accurately reflect transcript induction. Using the

**Table 1** Genes in cultured HOSE cells up-regulated by treatment with IL-1 $\alpha$ , based upon EUROSTERONE custom microarray analysis (mean fold change  $\pm$  S.E.M.)

	Genebank no.	Name	Fold change	P value
	XM031289	Interleukin-8	+3.72 $\pm$ 0.46	<0.01
	M14584	Interleukin-6	+4.93 $\pm$ 1.20	<0.01
	J00271	Metallothionein-IIA	+2.49 $\pm$ 0.59	<0.05
	M69043	NF $\kappa$ B inhibitor $\alpha$	+2.43 $\pm$ 0.55	<0.05
	AH006349	11 $\beta$ HSD type 1	+1.83 $\pm$ 0.12	0.01
	S78694	Lysyl oxidase	+2.11 $\pm$ 0.43	<0.05

**Table 2** Genes in cultured HOSE cells down-regulated by treatment with IL-1 $\alpha$ , based upon EUROSTERONE custom microarray analysis (mean fold change  $\pm$  S.E.M.)

	Name	Fold change	P value
<b>Genebank no.</b>			
Y13467	PPARbp	$-1.51 \pm 0.06$	0.01
AH005567	GnRH receptor	$-1.29 \pm 0.06$	<0.05
AF003341	Aldehyde dehydrogenase 1 soluble	$-2.60 \pm 0.09$	<0.05
S45679	3 $\beta$ HSD1	$-2.17 \pm 0.12$	<0.05
M64497	Nuclear receptor subfamily 2 group F member 2 (COUP-TF II)	$-1.36 \pm 0.08$	<0.05
I47162	Aldehyde dehydrogenase 10	$-1.24 \pm 0.06$	<0.05

same RNA preparation used for microarray analysis, gene-specific PCR was performed on a selection ( $n=7$ ) of the genes modulated by IL-1 $\alpha$ .

This analysis confirmed the reliability of the microarray data: all the genes tested showed the predicted shifts in expression, and to a generally comparable degree (Table 3).

#### Responsiveness to glucocorticoid

Non-steroidogenic genes induced by IL-1 $\alpha$  were deemed by definition to be 'inflammatory' and therefore were predicted to be responsive to cortisol, the metabolic product of 11 $\beta$ HSD1. This hypothesis was tested on LOX (an enzyme crucial to the deposition of mature collagen and elastin), showing reversal of IL-1 $\alpha$ -stimulated LOX mRNA by cortisol in HOSE cell cultures from three out of three patients (Fig. 3).

## Discussion

We demonstrate that cultured HOSE cells express all the genes required to synthesise *de novo* steroids in the C21-pregnane and C19-androstane series. They also express steroid dehydrogenase, hydroxylase and conjugation/deconjugation enzymes that endow the capacity to reversibly activate or inactivate multiple steroidal substrates. Critically, however, they lack detectable CYP19,

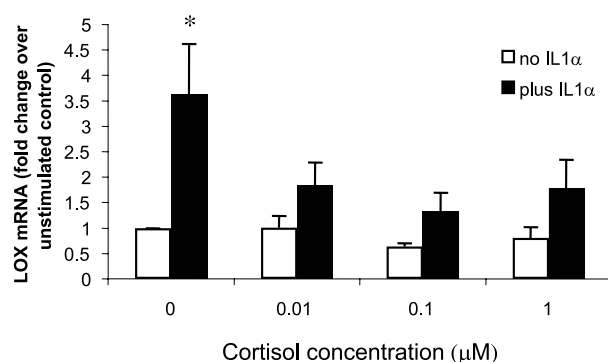
necessary for androgen aromatisation to oestrogen, and CYP21, required for C-21 hydroxylation and production of glucocorticoids.

This custom microarray analysis powerfully reinforces previous reports that human (Rembiszewska & Brynczak 1985) and sheep (Murdoch *et al.* 1999) OSE cells have limited steroid biosynthetic potential. It was not feasible to verify this conclusion exhaustively at the metabolic level. However, consistent with absence of CYP19 and presence of 11 $\beta$ HSD1 gene expression defined by the microarray, we confirmed absence of measurable aromatase enzyme activity (data not shown) but presence of 11-oxoreductase (Yong *et al.* 2002) in cultured HOSE cells. This, combined with the verification of selected genes by quantitative RT-PCR (Table 3), provides a strong measure of confidence in the validity of the present microarray dataset.

Exposure of HOSE cells to IL-1 $\alpha$ , as a surrogate inflammatory stimulus, was expected to cause pronounced changes in the steroidogenic expression profile of HOSE cells. In agreement with our previous finding (Yong *et al.* 2002), 11 $\beta$ HSD1 was stimulated by the cytokine. However, only one other steroidogenic gene was affected: 3 $\beta$ HSD1, which was suppressed. 3 $\beta$ HSD1 plays a critical role in steroid interconversion through catalysing the formation of 'mature'  $\Delta^4$ -3-oxo-C21 and -C19 products (progesterone, androstenedione) from immediate  $\Delta^5$ -3-hydroxy precursors (pregnenolone, dehydroepiandrosterone). Thus, at least during exposure to inflammation such

**Table 3** Subset of IL-1 $\alpha$ -regulated genes randomly selected and validated by quantitative real-time PCR on the same samples subjected to microarray analysis (mean fold change  $\pm$  S.E.M.)

	Name	Fold change	P value
<b>Genebank no.</b>			
M14584	Interleukin-6	$+20.93 \pm 10.66$	<0.05
M69043	NF $\kappa$ B inhibitor $\alpha$	$+4.61 \pm 0.98$	<0.05
AH006349	11 $\beta$ HSD type 1	$+12.94 \pm 3.89$	<0.01
S78694	Lysyl oxidase	$+3.18 \pm 0.31$	<0.01
Y13467	PPARbp	$-1.35 \pm 0.13$	<0.05
AF003341	Aldehyde dehydrogenase 1 soluble	$-4.74 \pm 0.53$	<0.01
S45679	3 $\beta$ HSD1	$-4.28 \pm 0.37$	<0.01



**Figure 3** Glucocorticoid-suppression of IL-1 $\alpha$ -induced LOX mRNA expression in cultured HOSE cells. Real-time PCR was used to determine gene expression changes due to IL-1 $\alpha$  and cortisol in primary HOSE cultures derived from three women. IL-1 $\alpha$  significantly increased expression of LOX mRNA ( $P < 0.05$ ). Cortisol alone had no effects; however, at all doses tested, it prevented the stimulatory effects of IL-1 $\alpha$  ( $P < 0.05$  as compared with IL-1 $\alpha$  alone). \*Significant difference,  $P < 0.05$ .

as encountered during ovulation, the HOSE is likely to function less as a *de novo* steroid hormone-producing tissue than one of steroid transformation.

11 $\beta$ HSD1 and 3 $\beta$ HSD1 are members of the SDR gene family, which encode enzymes that reversibly metabolise lipid-signalling molecules such as steroids, retinoids and prostaglandins (Oppermann 2001). Other SDR probes in the microarray included ALDH10 and ALDH1 soluble, both of which were expressed in HOSE cells and down-regulated by treatment with IL-1 $\alpha$ . ALDH isoforms constitute a large family of enzymes with the general reaction of catalysis of oxidation of long-chain aliphatic aldehydes to fatty acids, but may also act on steroid substrates. For instance, yeast SDRs FOX2 and AYR1 are active on long- and short-chain (3R)-hydroxyacyl-CoA substrates (Qin *et al.* 1999) and 1-acyl dihydroxyacetone phosphate (Athenstaedt & Daum 2000) respectively, but display surprisingly robust 17 $\beta$ HSD activity on dehydroepiandrosterone (DHEA)/androstenediol (Vico *et al.* 2002); some long-chain fatty acyl substrates may fold to adopt a steroid-like configuration on enzyme binding. The function of ALDHs also include reversible oxidation/reduction of retinol and retinal (Duester 2000), providing a mechanism for gating access of these ligands to RAR's. We found RAR $\alpha$ , RAR $\beta$ 4 and RAR $\gamma$  genes expressed in HOSE cells, underscoring the likelihood that ligand-activated RAR signalling is important to their development and survival *in vivo* (Um *et al.* 2001).

The presence of GR supports our central hypothesis that glucocorticoid fulfils anti-inflammatory signalling functions in the HOSE (Rae *et al.* 2004). This last study used quantitative RT-PCR to distinguish weak up-regulation of GR $\alpha$  mRNA by IL-1 $\alpha$ , which was not resolved by the microarray analysis used here, presumably due to its relative insensitivity.

The expression of PPAR $\delta$ , PPAR  $\gamma$ 2, PPAR co-activator 1 and PPAR-binding protein, and down-regulation of PPARbp by IL-1 $\alpha$ , is of special interest in view of the inferred overlaps between glucocorticoid and PPAR signalling (e.g. Lemberger *et al.* 1996, Johnson *et al.* 1999). However, many studies addressing PPAR function have depended on the thiazolidinedione (TZD) group of drugs such as effectors; this area may need to be reinterpreted in the light of a recent suggestion that TZDs primarily target a mitochondrial protein (Colca *et al.* 2004). PPARbp (also PBP) was identified as a coactivator for PPAR $\gamma$  but also binds to PPAR $\alpha$ , RAR $\alpha$ , RXR and TP $\beta$ 1 (Zhu *et al.* 1997), and a role in thyroid hormone signalling has been emphasised (Misra *et al.* 2002). In addition, over-expression of PPARbp has been reported in one-quarter of breast cancers examined, indicating a role in mammary epithelial differentiation and cancer development (Zhu *et al.* 1999) that may translate to the ovary. Suppression of PPARbp by IL-1 $\alpha$  is novel, and possibly mediated via nuclear factor  $\kappa$ B (NF $\kappa$ B), since there is a putative binding site for NF $\kappa$ B on the PPARbp gene (Zhu *et al.* 1999).

The presence of ER $\alpha$  and  $\beta$ , AR and PR confirms HOSE cells as sex-steroid targets, underscored by the detection of Hsp90, a molecular chaperone with an established function in the stabilisation of non-liganded nuclear receptors (Cadepond *et al.* 1991). The relative importance of ER $\alpha$  and ER $\beta$  in mediating cytoproliferative and other effects of oestrogen on HOSE cells is unknown but both are expressed (Hillier *et al.* 1998, Li *et al.* 2003). Notably, although steroid sulphatase was expressed, oestrone sulphotransferase was not. Thus HOSE cells may be exposed to locally enhanced levels of oestrogen by activation from oestrone sulphate, which circulates as the most abundant oestrogen in blood (Loriaux *et al.* 1971). Onward metabolism of oestrone to oestradiol is also predicted by the pattern of 17 $\beta$ HSD isoforms expressed. In support of this suggestion, in studies examining the role of oestrogens in normal OSE and malignant OSE cells, oestrone was found to be as effective as oestradiol in promoting cell proliferation (Syed *et al.* 2001); data presented here may explain this finding. The presence of AR in HOSE is in agreement with the pro-proliferative/anti-apoptotic effect of androgen on a virally transformed HOSE cell line reported by Edmondson *et al.* (2002). The presence of PR agrees with the anti-proliferative (Karlán *et al.* 1995, Murdoch & Van Kirk 2002) and anti-inflammatory (Rae *et al.* 2004) effects of progesterone that others have described. Additionally, we note expression of the membrane-bound progesterone receptor, providing a potential alternative route of progesterone action on these cells.

Other nuclear receptors expressed in HOSE cells include oestrogen-related receptors (ERR) $\alpha$  and ERR $\beta$ , which share a DNA-binding domain with ER $\alpha$  but are not activated by classical oestrogens. Their function in



HOSE cells is yet to be defined but, at least for  $ERR\alpha$ , a role in fatty acid metabolism is possible (Sladek *et al.* 1997). HOSE cells also express  $TR\alpha 2$  and  $TR\beta$ . To our knowledge, no published reports exist of thyroid hormone action on HOSE cells. However, epidemiological links between development of ovarian cancer, inflammation of the ovarian surface and hyperthyroidism have been previously established (Ness *et al.* 2000). In rats, direct action of thyroid hormones on the ovary has been discussed (Hori *et al.* 1992); studies in women have pointed to thyroid insufficiency as a possible cause of infertility (Gerhard *et al.* 1991, Raber *et al.* 2003). Further studies to elucidate the signalling function of thyroid hormone in normal and diseased HOSE cells are therefore urgently required.

Two genes in the array other than  $3\beta$ HSD1,  $ALD1$  soluble,  $ALD$  10 and  $PPARbp$  were significantly down-regulated by  $IL-1\alpha$  treatment. Expression of gonadotrophin-releasing hormone receptor ( $GnRH-R$ ) has previously been demonstrated in HOSE cells, for which  $GnRH$  is anti-proliferative *in vitro* (Kang *et al.* 2000). The functional significance of its suppression by  $IL-1\alpha$  remains unknown. The orphan steroid/retinoid receptor nuclear receptor subfamily 2 group F member 2 (also known as chicken ovalbumin upstream promoter transcription factor II-COUP TF II) was also down-regulated by treatment with  $IL-1\alpha$ . We are unaware of any previous report of this gene being expressed in the ovary. Its spatiotemporal expression pattern during mouse development suggests involvement in angiogenesis (Pereira *et al.* 1999). Such a role could be relevant to post-ovulatory tissue remodelling in the ovary. Importantly, it has been suggested that this gene may be a potential therapeutic target in disease conditions such as cancer where aberrant angiogenesis is a hallmark of disease progression (Pereira *et al.* 1999).

Treatment with  $IL-1\alpha$  stimulated the expression of five non-steroidogenic genes in the array, other than  $11\beta$ HSD1: metallothionein-IIA ( $MT-IIA$ ), previously implicated in protection against the oxidative stress generated by the inflammation associated with ovulation (Espey *et al.* 2003);  $IL-6$  and  $IL-8$ , established components of the ovulation-associated inflammatory cascade;  $LOX$ , responsible for the final catalysis of collagen formation and hence ECM deposition (Harlow *et al.* 2003); and  $NF\kappa B$  inhibitor  $\alpha$ , expression of which is associated as a downstream consequence of  $NF\kappa B$  signalling, which is in turn associated with inflammatory signalling. It has not escaped our attention that all these  $IL-1\alpha$ -responsive genes are potentially regulated by glucocorticoids in HOSE cells. Thus, cortisol dose-dependently enhances  $IL-1\alpha$ -stimulated  $11\beta$ HSD1 expression in HOSE cells (Rae *et al.* 2004); tumour-necrosis factor- $\alpha$  ( $TNF\alpha$ )-induced expression of  $IL-8$  and  $IL-6$  mRNA in human fibroblasts is suppressed by dexamethasone (Tobler *et al.* 1992);  $MT-IIA$  possesses a GR response element and is inducible by glucocorticoids (Karin *et al.* 1984);  $LOX$  enzyme

activity in rat skin is inhibited by glucocorticoids (Counts *et al.* 1986), and as shown here, cortisol suppresses  $IL-1\alpha$ -induced  $LOX$  mRNA expression in HOSE cells. This leads us to suggest that induction by  $IL-1\alpha$  of  $11\beta$ HSD1, augmented by cortisol, might serve to generate the glucocorticoid required to induce/repress anti-/pro-inflammatory gene responses *in vivo* (Rae *et al.* 2004). Moreover, through augmenting  $IL-1\alpha$ -stimulated  $11\beta$ HSD1 activity, cortisol would sustain its own regeneration and thereby amplify GR-mediated anti-inflammatory signalling in the postovulatory HOSE (Rae *et al.* 2004). However, although cortisone is the classic  $11\beta$ HSD1 substrate, we cannot rule out the possibility that other substrates for this enzyme are present in HOSE cells.

Caveats to this study are that we have provided a restricted gene expression profile for cultured cells at a fixed time of stimulation. Concerning the first point, the central weakness of microarray studies is that the significance of individual levels of gene expression cannot easily be addressed, nor is it possible to ascertain whether mRNA levels accurately reflect polypeptide expression. However, we feel that induced changes in gene expression levels such as those observed here, and confirmed by PCR, are likely to reflect changes in polypeptide product levels irrespective of translational efficiency. Further, the core gene set comprehensively covered the conventional steroid biosynthetic pathway module, which was the main purpose of the exercise. We duly acknowledge that sensitivity of microarray may permit false-negative data, i.e. apparent lack of expression of a gene, as compared with metabolic profiling. However, as a site of major endocrine-related gynaecological disease – including ovarian cancer and endometriosis – there is an urgent need to catalogue, characterise and comprehend the expression profile of genes in HOSE cells involved in steroid signalling, in order to define new diagnostic and therapeutic targets. Regarding the second point, the only way that sufficient HOSE cells for array analysis can be obtained from individual patients is to biopsy and propagate *in vitro*, as described here. The extent to which HOSE cells propagated for 3 weeks *in vitro* reflect an *in vivo* phenotype is difficult to assess. However, a benefit of primary culture is that any confounding patient-to-patient variable should have been minimised, as suggested by the remarkably uniform response to  $IL-1\alpha$  given by the cells from all five patients. A strength of this study is that it compared 'like with like', that is, all samples were primary cell cultures, and each had an untreated and a treated component, identical to each other in all other ways. Selection of a suitable control in gene profiling studies in HOSE has recently been shown to dramatically effect data and interpretation (Zorn *et al.* 2003). Thirdly, the use of a 48 h period of stimulation by fixed-dose  $IL-1\alpha$  was a compromise, based on previous evidence that  $11\beta$ HSD1 mRNA and enzyme activity in HOSE cells are maximally stimulated under these conditions. (Yong *et al.* 2002, Rae *et al.*

2004). The trade-off for this restriction could be that earlier gene changes might have been missed. These criticisms aside, the dataset presented provides novel and revealing information on this poorly understood specialised human ovarian cell type.

In conclusion, we have used a custom oligonucleotide microarray to define the steroidogenic potential of cultured HOSE cells and investigate changes associated with inflammatory stimulation through exposure to IL-1 $\alpha$ . Results suggest that the HOSE is principally a site of steroid metabolism and reception as opposed to *de novo* steroid hormone synthesis. Intriguingly, the only steroidogenic gene detectably up-regulated by IL-1 $\alpha$  is 11 $\beta$ HSD1. Non-steroidogenic genes affected include pro-/anti-inflammatory genes whose expression is inhibited/augmented by corticosteroid. Since the steroidogenic function of 11 $\beta$ HSD1 is to regenerate cortisol from cortisone, these results support the hypothesis that locally produced glucocorticoids play significant roles in HOSE cell function, including a possible anti-inflammatory action around the time of ovulation.

### Note added in proof

During preparation of this article for publication, COUP-TF II has been found to be downregulated in ovarian cancer cells (Lee *et al.* 2004).

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